Supplemental Data

Quantitative Cell-based Protein Degradation Assays to Identify and Classify Drugs that Target the Ubiquitin-Proteasome System

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Running Title: Quantitative cell-based degradation assay

Supplemental Methods.

Recombinant protein expression and purification. All buffers were prepared in deionized water and filtered through a 0.2 μm filter. For recombinant expression of mouse p97, *E. coli* BL21 (DE3) containing the plasmid RDB2120[1] was grown in LB medium containing 50 μg/L ampicillin with shaking at 37 °C to an OD_{600} of 0.5. The cell culture was cooled down to 22°C and induced with 1 mM IPTG and harvested 8-10 hours later by centrifugation. The cell pellet (approximately 6 g from 2L) was suspended in 30 mL lysis buffer (100 mM Tris, pH 7.4, 500 mM KCl, 5 mM $MgCl_2$, 20 mM imidazole, 5% glycerol, 2 mM β-mercaptoethanol, protease inhibitor tablet (Roche)). The cells (held in an ice bath) were lysed by six 30-second pulses of sonication, separated by 2 min intervals. The lysate was centrifuged at 20,000g for 45 min at 4 °C, and the resulting supernatant was loaded onto a Ni-NTA column (5 mL suspension, pre-equilibrated with wash buffer (50 mL, 50mM HEPES, pH 7.4, 150 mM KCl, 5 mM $MgCl_2$, 20 mM imidazole)) and incubated at 4 °C with rotation for 30 min. The column was then flushed with wash buffer (100 mL) and His_6-tagged p97 was eluted by stepwise application of 10 mL of imidazole elution buffer (50 mM, 100 mM, 150mM, 200 mM, or 250mM imidazole in wash buffer). Fractions from the 200 mM and 250 mM imidazole steps were combined and concentrated with an Amicon Ultra-15 centrifugal filter unit (NMWL = 100 kDa). The mixture (0.5 mL of 20 mg/mL) was then fractionated with a gel filtration column (Tricorn™ Superdex™ 200, GE Healthcare), eluted with GF buffer (20 mM HEPES, pH 7.4, 250 mM KCl, 1mM $MgCl_2$) at 0.5 mL/min flow rate, and fractions corresponding to an apparent molecular weight of 500-600 kDa were collected and analyzed by 4-12% SDS-PAGE to evaluate purity (Invitrogen). Fractions that contained p97 of ≥95% purity were concentrated to 5 mg/mL, exchanged into storage buffer (20 mM HEPES, pH 7.4, 250 mM KCl, 1mM $MgCl_2$, 5% glycerol, 1mM DTT), aliquoted, frozen in liquid nitrogen, and stored at -80 °C.

Ub^{G76V}-GFP degradation assay. Two GFP images with 100 ms exposure time per well were acquired and the average GFP intensity per area of a HeLa cell was determined by using MetaXpress software. Mean GFP intensity of 300-500 cells was calculated using Excel. Normalized GFP intensity was calculated using the following formula: (Test compound – Background)/(Basal GFP intensity – Background). Where: Test compound is defined as Mean GFP intensity of Ub^{G76V}-GFP-expressing cells treated with the test compound. Background is defined as background GFP intensity of HeLa cells. Basal

GFP intensity is defined as mean GFP intensity of Ub^{G76V}-GFP-expressing cells treated with DMSO. The degradation rate constant (k) was obtained from the slope of the linear range of plotting Ln (Normalized GFP intensity) versus time ranging from 90 to 180 min. The percent of remaining k for each compound is calculated using the following formula: (Test compound/DMSO control) * 100. Where: Test_compound is defined as k determined from wells containing test compound, DMSO control is defined as k determined from wells containing DMSO. IC_{50} values were calculated from fitting the percentage of remaining k (%k) with various concentrations of compounds to a Langmuir equation [%k =100/(1 + [Compound]/IC_{50})] by non-linear regression analysis using the JUMP IN program. The result was expressed as mean +/- standard error.

ATPase assay. Assay Buffer (20 µL of 2.5x concentration, where 1x = 50 mM Tris pH 7.4, 20 mM $MgCl_2$, 1 mM EDTA, 0.5 mM TCEP) was dispensed into each well of a 96 well plate. Purified p97 (25 µL of 50 µM) was diluted in 975 µL of 1x Assay Buffer and 10 µL was dispensed in each well. Test compound (10 µL) or 5% DMSO (10µL) was then added to each well and the plate was incubated at room temperature for 10 min. The ATPase assay was carried out by adding to each well 10 µL of 500 µM ATP (pH 7.5), incubating at room temperature for 60 min, and then adding 50 µL Kinase Glo Plus reagent (Promega) followed by a final 10 min incubation at room temperature in the dark. Luminescence was read on an Analyst AD (Molecular Devices). Compounds were assayed at a range of concentrations (0, 0.048, 0.24, 1.2, 6, 30 µM) in triplicate. The percent of remaining activity for each reaction was calculated using the following mathematical expression: ((Test Compound-High Control)/(Low Control-High Control)) * 100. Test_Compound is defined as wells containing test compound, Low_Control is defined as wells containing DMSO, High_Control is defined as wells containing no p97 protein. IC_{50} values were calculated from fitting the percentage of remaining activity (%RA) with various concentrations of compounds to a Langmuir equation [%RA =100/(1 + [Compound]/IC_{50})] by non-linear regression analysis using the JUMP IN program. The result was expressed as mean +/- standard error.

For assays with Myriad 12 and 19, 100 µL of biomol green reagent (Enzo Life Sciences) was added to each well instead of kinase Glo Plus (Promega) and absorbance at 630 nm was measured. This was done because these compounds interfered with luciferase activity.

Human 26S proteasome activity assay. Inhibition of hydrolysis of the fluorogenic proteasome substrate (succinyl-Leu-Leu-Val-Tyr- AMC, 60 µM, Boston Biochem) by purified human 26S proteasome (1 nM) from Enzo life science in Assay Buffer containing ATP (50 mM Tris pH 7.4, 20 mM $MgCl_2$, 1 mM EDTA, 0.5 mM TCEP, 100 µM ATP). Fluorescence intensity was monitored every 5 min over 60 min. IC_{50} values were calculated from fitting the percentage of remaining activity (%RA) with various concentrations of compounds to a Langmuir equation [%RA =100/(1 + [Compound]/IC_{50})] by non-linear regression analysis using JUMP IN program. The result was expressed a mean ± standard error.

Western blot analysis. Cells were seeded on 6 well plates (50000 cells) and grown for 16h. For the accumulation assay, cells were treated with compounds (10 µM) for 2h. For

the degradation assay, cells were first treated with MG132 (4 μM) for 1h and washed with PBS (100μL) twice. DMEM containing 2.5% FBS, cycloheximide (30 μg/mL) and the test compound was added into the well. Cells were harvested after a 2h chase by trypsinization and centrifugation at 5000 xg for 4 min at 4 ^{o}C. Cell pellets were resuspended in ice-cold PBS (750 μL) then centrifuged. Cell pellets were immediately frozen in a -80 ^{o}C freezer and lysed in ice-cold Buffer A (50 mM Tris, pH 7.4, 1 % Triton-X100, 200 mM NaCl, 1 mM EDTA, 2 mM $MgCl_2$, 5 mM TCEP, 10 mM NEM, protease inhibitor tablet (Roche), and Halt Phosphatase Inhibitor Cocktail (Thermo Scientific)). Soluble cell extract (100 μL) was mixed with SDS-sample buffer (4x) and heated at 90 ^{o}C for 5 min. After centrifugation at 3000 xg for 30 sec, sample (25 μg) was loaded on a 4-12% SDS-PAGE. Proteins were transferred to nitroncellulose membranes and stained with Ponceau S. GFP-based reporters were detected with anti-GFP antibody and luciferase-based reporters were detected with anti-Luciferase antibody.

Supplemental Reference:

1 DeLaBarre, B., and Brunger, A. T. (2003) *Nature structural biology*. **10**, 856-863.

2 Dalal, S., Rosser, M. F, Cyr, D. M., and Hanson, P. I. (2004) *Mol. Biol. Cell*. **15**, 637-648.

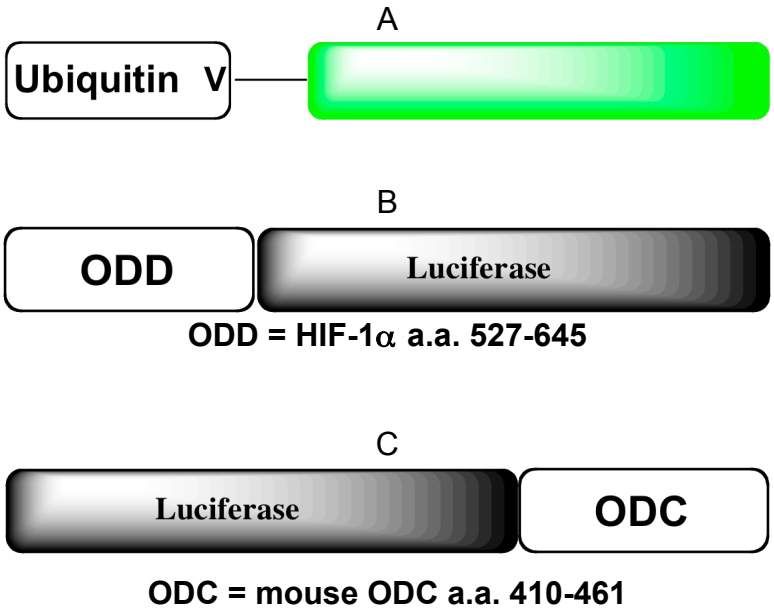
3 DeLaBarre, B., Christianson, J. C., Kopito, R. R., and Brunger, A. T. (2006) *Mol. Cell*. **22**, 451-462.

4 Kimbrel, E. A., Davis, T.N., Bradner, J.E., and Kung, A.L. (2009) *Molecular Imaging*. **8**, 141-147.

5 Dantuma, N. P., Lindsten, K., Glas, R., Jellne, M., and Masucci, M. G. (2000) *Nat Biotechnol.* **18**, 538-543.

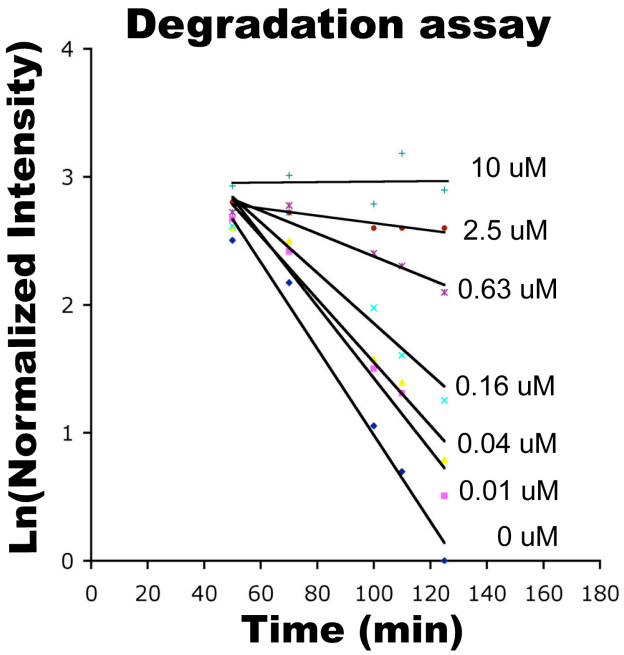
Supplemental Figures

Figure 1. The GFP- and luciferase-based UPS reporters.



(A) Ub^{G76V}-GFP: a ubiquitin fusion degradation (UFD) pathway reporter. (B) ODD-Luc: a $CRL2^{VHL}$ and proteasome substrate derived from the oxygen-dependent destruction domain of HIF-1α. (C) Luc-ODC: an ubiquitin-independent proteasome reporter derived from the ubiquitin-independent degradation domain of ornithine decarboxylase.

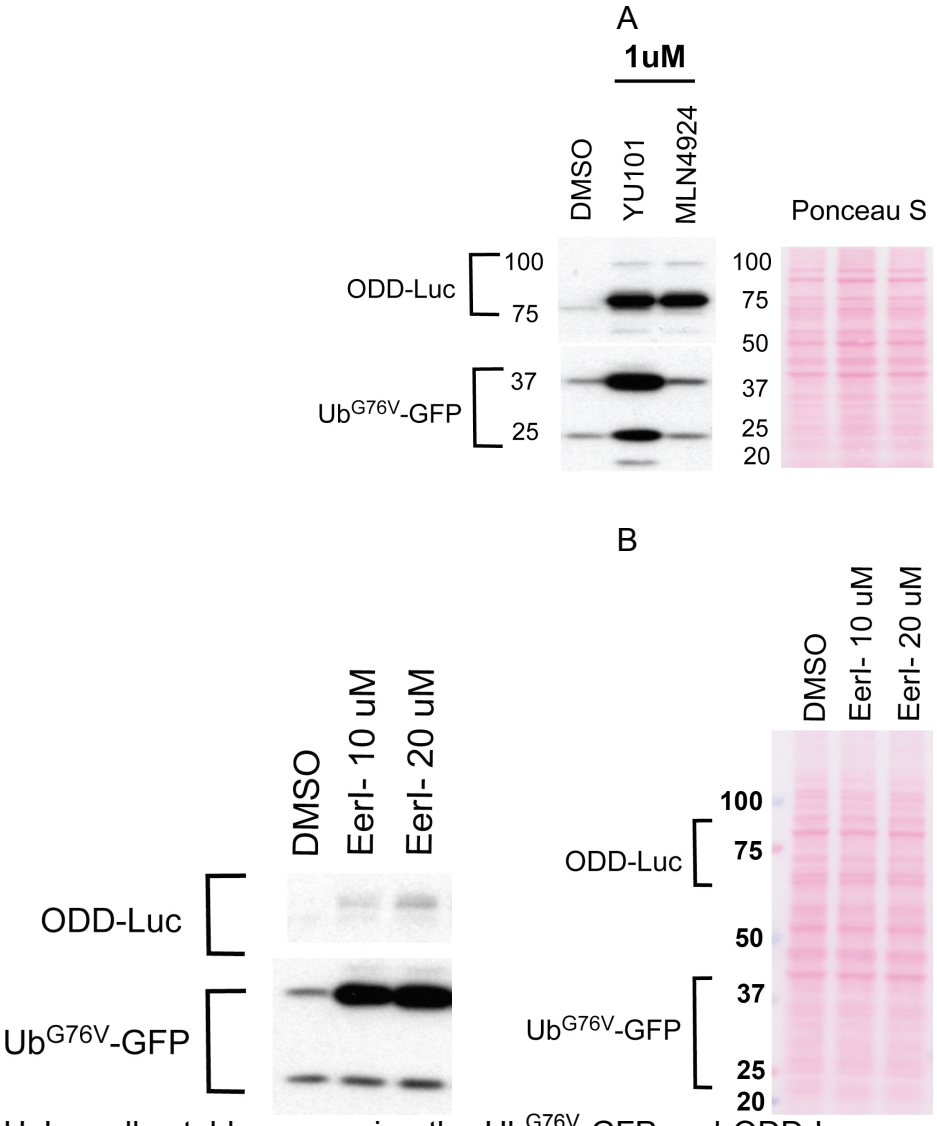
Figure 2. A representative figure for determining the half-maximal inhibitory concentration (IC_{50}) of MG132 in the Ub^{G76V}-GFP turnover assay.



[MG132] (μM)	Rate constant k (1/min)	%RA
0	0.0337	100
0.010	0.0273	81.0
0.039	0.0246	73.0
0.156	0.0197	58.5
0.625	0.0089	26.4
2.5	0.0029	8.6
10	0.0021	6.2

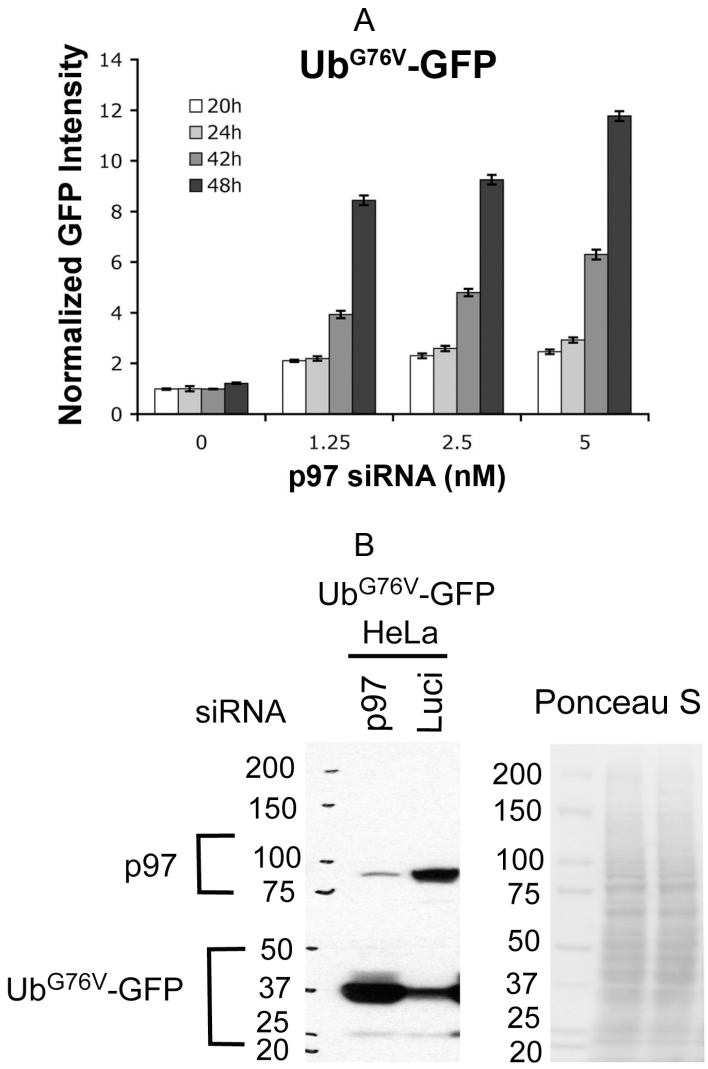
Ln (Normalized Reporter Intensity) versus time was plotted for a series of concentrations of MG132 and the degradation rate constant (k) was obtained from the slope. IC_{50} values were calculated from fitting the percentage of remaining activity (%RA) at various concentrations of compound to a Langmuir equation [%RA =100/(1 + [Compound]/IC_{50})] by non-linear regression analysis using the JUMP IN program. The result was expressed as mean +/- standard error, where %RA = 100 x (k from a certain concentration of MG132 divided by k from the DMSO treated sample)

Figure 3. Western blot assays to confirm accumulation of reporters and inhibition of reporter turnover.



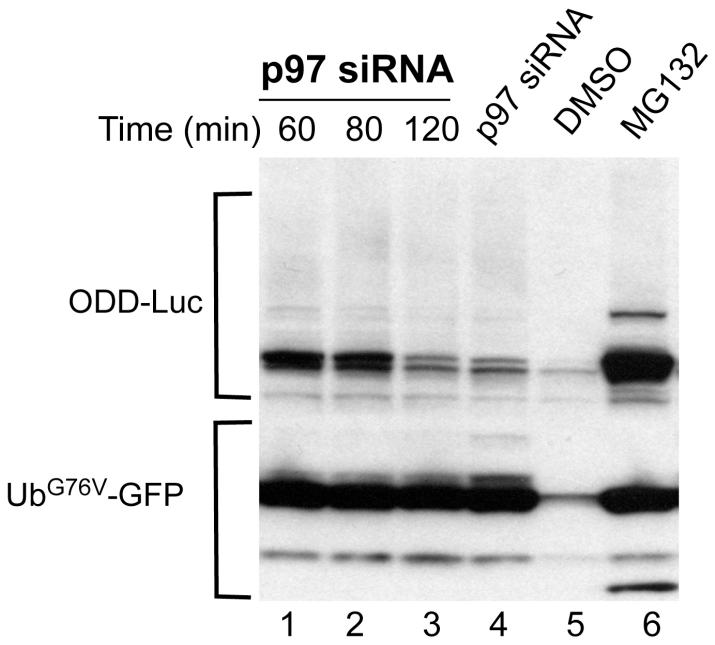
HeLa cells stably expressing the Ub^{G76V}-GFP and ODD-Luc reporters were grown in 6 well plates. (A) Cells were treated with YU101 (1 μM) or MLN4924 (1 μM) for 2 h and analyzed for their content of Ub^{G76V}-GFP and ODD-Luc by immunoblotting. The Ponceau S stain of the filter is shown as a control for equal loading. (B) Cells were treated with MG132 (4 μM for 1 h) to accumulate reporter, washed, and exposed to the indicated test compounds plus CHX to initiate a chase. After 2h, cells were harvested and evaluated by immunoblotting for the remaining ODD-Luc and Ub^{G76V}-GFP. The Ponceau S-stained filters serve as loading controls.

Figure 4. Accumulation of Ub^{G76V}-GFP in cells depleted of p97 by siRNA.



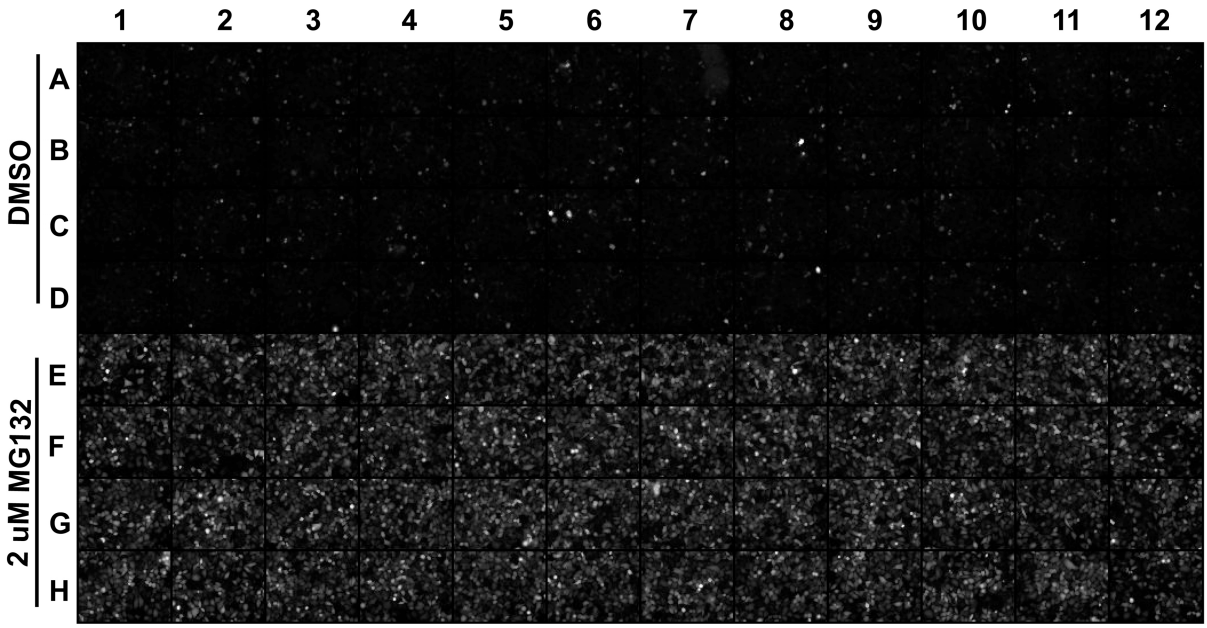
(A) HeLa cells expressing Ub^{G76V}-GFP were grown in a 96-well plate and transiently transfected with p97 siRNA (1.25 - 5 nM) or control siRNA for 20 - 48 h. Prior to imaging on an ImageXpress microscope, growth medium was replaced with PBS and GFP fluorescence was measured and normalized to the values obtained from cells transfected with control siRNA. (B) Ub^{G76V}-GFP-expressing HeLa cells were transiently transfected with control siRNA (5 nM) was analyzed by immunoblotting with p97 and GFP antibodies. The Ponceau S-stained filter serves as a loading control.

Figure 5. Validation of p97-dependent and independent reporters by siRNA combined with western blotting



Western blot analysis of cells expressing both Ub^{G76V}-GFP and ODD-Luc. Cells were transfected with p97 siRNA (lanes 1-4) or mock-transfected (lanes 5-6). Forty-eight h after siRNA transfection, cells were treated with MG132 (4μM) for 1 h, followed by a chase in CHX for 60 min, 80 min, or 120 min (Lanes 1 - 3). Lane 4 shows the basal level of ODD-Luc and Ub^{G76V}-GFP in p97-depleted cells not subjected to the MG132/cycloheximide chase protocol. Lanes 5 and 6 were the same as Lane 4, except that cells were treated with either DMSO or MG132 (10 μM, 2h) prior to lysis.

Figure 6 Ub^{G76V}-GFP HTS degradation assay



A representative image from a Ub^{G76V}-GFP degradation assay carried out on a 96-well plate and acquired on an ImageXpress automated microscope. Cells were treated with MG132 for 1 h, after which cells were washed with MG132-free medium and resupplied with fresh medium that either contained DMSO or 2 μM MG132. The image was acquired 3 hours later.

Supplemental Table

Table 1. Plasmids, Primers Cell lines and siRNAs used in this study

Plasmids

RDB number	Plasmid name	Vector	Source and Reference
2003	Murine p97-myc His	pcDNA4.1TO (Invitrogen)	P.I. Hanson[2]
2004	Murine p97 E578Q -myc	pcDNA4.1TO (Invitrogen)	PI Hanson[2]
2120	Murine p97-pET28a	pET28a (Novagen)	A. T. Brunger[3]
2390	FUW-puro-LucODC-mCh-puro	FUW lentiviral plasmid	A. L. Kung[4]
2391	FUW-ODD-Luc-mCh-puro	FUW lentiviral plasmid	A. L. Kung[4]
2392	FUW-Luc-mCh-puro	FUW lentiviral plasmid	A. L. Kung[4]
2402	C522A-p97-PET28a/DH5a	pET28a (Novagen)	Made in this study
2405	QQmp97-myc His/pcDNA4.1TOsiRNA	pcDNA4.1TO (Invitrogen)	Made in this study

Primers used in this study

Name	Sequence
E305Q-F	ATCTTCATCGATCAGCTTGATGCCATTGCACC
E305Q-R	GGTGCAATGGCATCAAGCTGATCGATGAAGATG
E578Q-F	GTACTCTTCTTTGATCAGTTAGATTCAATTGCC
E578Q-R	GGCAATTGAATCTAACTGATCAAAGAAGAGTAC
C522A-F	CTATGGACCTCCTGGCGCTGGGAAAACTTTGTTGGCC
C522A-R	GGCCAACAAAGTTTTCCCAGCGCCAGGAGGTCCATAG

Cell lines used in this study

DTC number	Cell line name	Source and Reference
9	Ub^{G76V}-GFP/HeLa	M. G. Masucci and N. P. Dantuma[5]
20	293T	D. Baltimore
23	Ub^{G76V}-GFP/Luc-ODC/HeLa	Made in this study
24	Ub^{G76V}-GFP/Luciferase/HeLa	Made in this study
25	Ub^{G76V}-GFP/ODD-Luc/HeLa	Made in this study

siRNAs used in this study

Target Gene	Thermo Fisher Scientific catalog number
Luciferase	P-002099-01-20
p97/VCP	D008727060050
Target Gene	**Qiagen catalog number**
None	Negative Control siRNA (1027310)

Table 2. Summary of p97 effect on degradation of reporters

	Half-life (min)*			
Reporter	**Control siRNA**	**p97 siRNA**	**WT-p97**	**QQ-p97**
Ub^{G76V}-GFP	38 ± 1	1072 ± 116	38 ± 3	530 ± 60
ODD-Luc	22 ± 4 (0-20 min)** 110 ± 3 (20-120 min)**	14 ± 2 (0-20 min)** 76 ± 7 (20-120 min)**	47 ± 8	48 ± 5
Luc-ODC	137 ± 2	140 ± 2	116 ± 2	116 ± 6

* Measurements were carried out in duplicate, and variance is expressed as the standard deviation.

** Degradation of the reporter was biphasic. Degradation rates were estimated independently for each phase.

Table 3A. InhibitorSelect™ Protein Kinase Inhibitor Libraries I and II.

EMD. Cat. No. 539744 and 539745

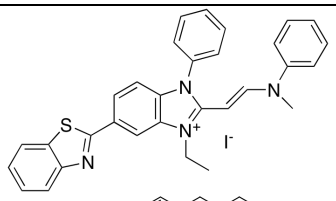
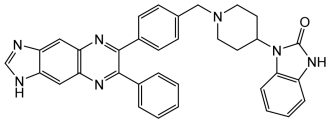
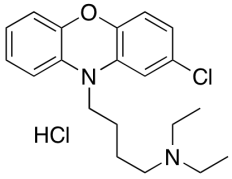
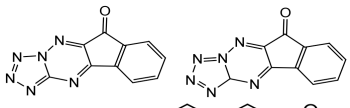
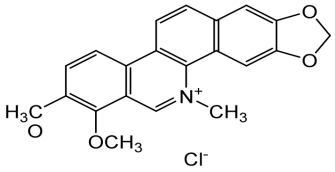
Well number	Description	PubChem Compound ID #	% Inhibition @ 12 μM
Library I			
A2	AG 1024	2044	23
A3	AGL 2043	9817165	32
A4	Akt Inhibitor IV	5719375	61
A5	Akt Inhibitor V, Triciribine	290486	0
A6	Akt Inhibitor VIII, Isozyme-Selective, Akti-1/2	10196499	99
A7	Akt Inhibitor X	16760284	61
A8	PDK1/Akt/Flt Dual Pathway Inhibitor	5113385	86
A9	Aurora Kinase Inhibitor II	6610278	23
A10	Bcr-abl Inhibitor	5311510	17
A11*	Bisindolylmaleimide I	2396	55
B2	Bisindolylmaleimide IV	2399	-9
B3	BPIQ-I	2427	21
B4	Chelerythrine Chloride	72311	88
B5	Compound 56	2857	25
B6	DNA-PK Inhibitor II	9860529	21
B7	DNA-PK Inhibitor III	9859309	-7
B8	PI-103	9884685	26
B9	Diacylglycerol Kinase Inhibitor II	657356	-4
B10	DMBI	5353593	29
B11	EGFR/ErbB-2 Inhibitor	9843206	19
C2	EGFR Inhibitor	9549299	39
C3	EGFR/ErbB-2/ErbB-4 Inhibitor	11566580	17
C4	Flt-3 Inhibitor	1048845	28
C5	Flt-3 Inhibitor II	11601743	28
C6	cFMS Receptor Tyrosine Kinase Inhibitor	11617559	17
C7	Gö 6976	3501	10
C8	Gö 6983	3499	23
C9	GTP-14564	3385203	27
C10	Herbimycin A, Streptomyces sp.	16760502	75
C11	Flt-3 Inhibitor III	11772958	13
D2*	IGF-1R Inhibitor II	9549305	53
D3	IRAK-1/4 Inhibitor	11983295	43
D4	JAK Inhibitor I	5494425	13
D5	JAK3 Inhibitor II	3795	27
D6	JAK3 Inhibitor IV	176406	83
D7	JAK3 Inhibitor VI	16760524	47
D8	Lck Inhibitor	6603792	44
D9	LY 294002	3973	45
D10	LY 303511	3971	-11
D11*	Met Kinase Inhibitor	9549297	100
E2*	PD 158780	4707	53
E3	PD 174265	4709	33
E4	PDGF Receptor Tyrosine Kinase Inhibitor II	5330548	5
E5	PDGF Receptor Tyrosine Kinase Inhibitor III	10907042	65

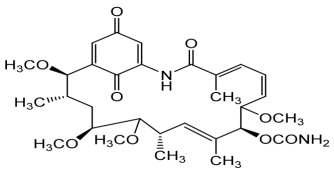
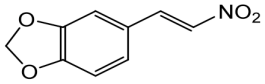
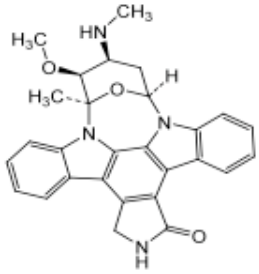
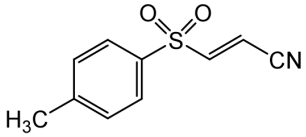
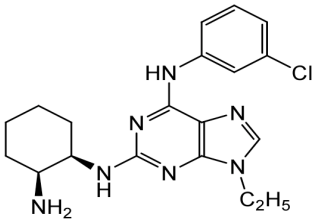
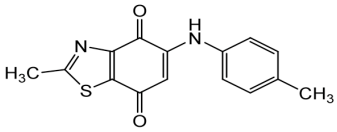
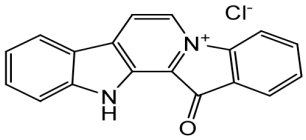
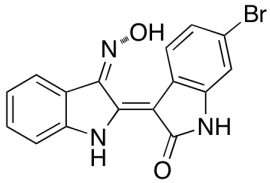
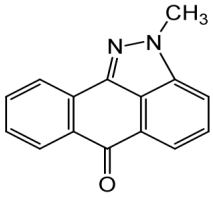
E6	PDGF Receptor Tyrosine Kinase Inhibitor IV	9797370	-1
E7	PDGF RTK Inhibitor	16760609	5
E8	PKR Inhibitor	6490494	13
E9	PKR Inhibitor, Negative Control	16760619	25
E10	PI 3-Kg Inhibitor	5289247	7
E11	PI 3-KbInhibitor II	5287855	3
F2	PP3	4879	5
F3	PP1 Analog II, 1NM-PP1	5154691	42
F4*	PKCbII/EGFR Inhibitor	6711154	76
F5	PKCb Inhibitor	6419755	40
F6	Rapamycin	16760631	65
F7	Rho Kinase Inhibitor III, Rockout	644354	23
F8	Rho Kinase Inhibitor IV	16760635	20
F9*	Staurosporine, N-benzoyl-	16760627	1
F10	Src Kinase Inhibitor I	1474853	18
F11	SU11652	5329103	2
G2*	Syk Inhibitor	6419747	100
G3	Syk Inhibitor II	16760670	26
G4	Syk Inhibitor III	672296	95
G5	TGF-b RI Kinase Inhibitor	447966	26
G6	TGF-b RI Inhibitor III	16079009	12
G7	AG 9	2063	21
G8	AG 490	5328779	10
G9	AG 112	5328804	1
G10	AG 1295	2048	2
G11	AG 1296	2049	29
H2	AG 1478	2051	16
H3*	VEGF Receptor 2 Kinase Inhibitor I	6419834	100
H4	VEGF Receptor Tyrosine Kinase Inhibitor II	9797919	35
H5	VEGF Receptor Tyrosine Kinase Inhibitor III, KRN633	9549295	1
H6*	VEGF Receptor 2 Kinase Inhibitor II	5329155	100
H7*	VEGF Receptor 2 Kinase Inhibitor III	5329098	87
H8	VEGF Receptor 2 Kinase Inhibitor IV	5329468	-3
H9	DNA-PK Inhibitor V	16760391	-10
H10	Aurora Kinase Inhibitor III	9549303	-16
H11	Staurosporine, Streptomyces sp.	451705	83
Library II			
A2	KN-62	16760529	9
A3	ATM Kinase Inhibitor	5278396	11
A4	ATM/ATR Kinase Inhibitor	6605258	32
A5	Alsterpaullone	5005498	8
A6	Alsterpaullone,	16760286	25
A7	Aloisine A, RP107	5326843	3
A8	Aloisine, RP106	3641059	26
A9	Aminopurvalanol A	6604931	31
A10	AMPK Inhibitor	11524144	3
A11	Aurora Kinase Inhibitor III	9549303	13
B2	Aurora Kinase/Cdk Inhibitor	16760303	4
B3	Indirubin-3′-monoxime	5326739	2

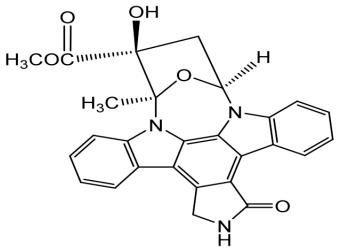
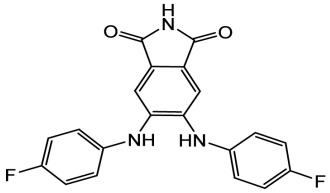
B4	BAY 11-7082	5353431	80
B5	Bohemine	2422	-18
B6	Cdk1 Inhibitor	5472558	44
B7	Cdk1 Inhibitor, CGP74514A	2794188	72
B8	Cdk1/2 Inhibitor III	5330812	-21
B9	Cdk1/5 Inhibitor	438981	-17
B10	Casein Kinase I Inhibitor, D4476	6419753	6
B11	Casein Kinase II Inhibitor III, TBCA	16760346	14
C2	Cdk4 Inhibitor	5330797	-6
C3	Cdk4 Inhibitor II, NSC 625987	3004085	-6
C4	Cdk4 Inhibitor III	481747	77
C5	Cdc2-Like Kinase Inhibitor, TG003	1893668	25
C6	Chk2 Inhibitor II	9969021	10
C7	Compound 52	2856	6
C8	Cdk2 Inhibitor III	6918386	0
C9	Cdk2 Inhibitor IV, NU6140	10202471	38
C10	Cdk/Crk Inhibitor	9549301	14
C11	ERK Inhibitor III	5339183	10
D2	ROCK Inhibitor, Y-27632	9797929	24
D3	ERK Inhibitor II, FR180204	11493598	20
D4	ERK Inhibitor II, Negative control	16760417	25
D5	Fascaplysin,Synthetic	73292	86
D6	GSK-3b Inhibitor I	4124851	98
D7	GSK-3b Inhibitor II	6539732	19
D8*	GSK-3b Inhibitor VIII	448014	87
D9*	GSK-3 Inhibitor IX	5287844	100
D10	GSK-3 Inhibitor X	6538818	9
D11	GSK-3b Inhibitor XI	10020713	-16
E2*	SU6656	5312137	100
E3	GSK-3 Inhibitor XIII	6419766	19
E4	Isogranulatimide	6419741	15
E5	IC261	3674	20
E6	IKK-2 Inhibitor IV	9903786	23
E7	Indirubin Derivative E804	6419764	10
E8*	JNK Inhibitor II	8515	39
E9	JNK Inhibitor, Negative Control	11665831	89
E10	JNK Inhibitor V	11422035	86
E11	JNK Inhibitor IX	16760525	29
F2	MK2a Inhibitor	11382492	30
F3	JNK Inhibitor VIII	11624601	29
F4	K-252a, Nocardiopsis sp.	490561	76
F5	Kenpaullone	3820	20
F6	KN-93	5312122	13
F7	MEK Inhibitor I	9951490	19
F8	MEK Inhibitor II	389898	7
F9	MEK1/2 Inhibitor	9549284	15
F10	MNK1 Inhibitor	11644425	71
F11	NF-κB Activation Inhibitor	509554	20
G2	p38 MAP Kinase Inhibitor III	6419739	46
G3	p38 MAP Kinase Inhibitor	4665	53

G4	PD 98059	4713	34
G5	PD 169316	4712	43
G6	SB220025	5164	19
G7	Purvalanol A	4987	20
G8	GSK3b Inhibitor XII, TWS119	9549289	11
G9	H-89, Dihydrochloride	5702541	21
G10	SB 202474, Negative control p38 MAPK inhibitor	5162	15
G11	SB 202190	5353940	19
H2	SB 203580	176155	-13
H3	HA 1077, Dihydrochloride Fasudil	16219471	-1
H4*	SB 218078	3387354	100
H5	SC-68376	5174	4
H6	SKF-86002	5228	17
H7	Sphingosine Kinase Inhibitor	16760659	25
H8	Staurosporine, Streptomyces sp.	451705	97
H9	STO-609	16760660	15
H10	SU9516	5289419	21
H11*	Tpl2 Kinase Inhibitor	9549300	100

Table 3B. Structure of 17 hits from the kinase inhibitor screen

Comp. #	PubChem CID #	Description	Structure
1	5719375	Akt Inhibitor IV	
2	10196499	Akt Inhibitor VIII	
3	16760284	Akt Inhibitor X	
4	5113385	PDK1/Akt/Flt	
5	72311	Chelerythrine Chloride	

6	16760502	Herbimycin A, Streptomyces sp.	
7	672296	Syk Inhibitor III	
8	451705	Staurosporine, Streptomyces sp.	
9	5353431	BAY 11-7082	
10	2794188	Cdk1 Inhibitor	
11	481747	Cdk4 Inhibitor III	
12	73292	Fascaplysin	
13	5287844	GSK-3 Inhibitor IX	
14	11665831	JNK Inhibitor, Negative Control	

15	490561	K-252a, Nocardiopsis sp.	
16	6711154	PKCbII/EGFR Inhibitor	
17	9549300	Tpl2 Kinase Inhibitor